PCT/EP 99/10320

EPO - DG 1

14. 02. 2000

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APPLICATION NUMBER: *60/113,989*
FILING DATE: *December 28, 1998*

REC'D 25 FEB 2000
WIPO PCT

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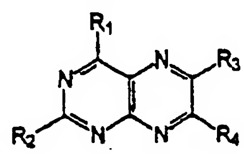
Certifying Officer

IMMUNOSUPPRESSIVE EFFECTS OF PTERIDINE DERIVATIVES

The invention relates to a pharmaceutical composition for the treatment of autoimmuno disorders or for the treatment and/or prevention of transplant-rejections and/or the treatment of inflammatory diseases comprising as active ingredient one or more pteridine derivatives having the general formula:



(I)

wherein:

R_1 and R_2 are independently amino, hydroxylamino, alkoxyamino, hydrazino, piperazino, N-alkylpiperazino, morpholino, mono- and diarylamino, (wherein the aryl group may be the same or different) mono- and dialkylamino (wherein the alkyl group may be the same or different), mono- and diarylalkylamino (wherein both groups may be the same or different), cycloalkylamino (such as cyclopropylamino, cyclobutylamino, cyclopentylamino, cyclohexylamino), alkoxy, mercaptoalkyl . The alkyl group may contain 1 to 7 carbon atoms and may be branched, cyclized and may be oxidized.

R_3 : unsubstituted , monosubstituted or disubstituted aryl group (wherein the substituent may be , but not limited to, halogen, alkoxy, alkyl), aryl group bond to the pteridine ring via a saturated or unsaturated alifatic spacer which may be halogenated or hydroxylated, aliphatic substituent which may contain ether function, alcohol function, substituted or unsubstituted amino functions.

R_4 : hydrogen, alkyl, alkoxy, substituted or unsubstituted aryl.

The invention further relates to combined pharmaceutical preparations comprising one or more pteridine derivates and one or more known immunosuppressant, and to a group of novel pteridine derivates as such.

Further the invention is also related to a method for the treatment of autoimmuno disorders and/or of transplant-rejections and/or inflammatory diseases.

The invention further relates to a method for the preparation of the above mentioned pteridine derivatives and the the pteridine derivatives as such.

Several pteridine derivates are known in nature and used in the preparation of medicines, for example as described in EP-A-108 890. Other medical uses of derivates of pteridine are described in WO 95-31987 as NO-synthase inhibitors for example for the treatment of diseases caused by a high nitrogen monoxide level. Further, WO-95-32203 describes also the use of tetrahydropteridine derivates as NO-synthase inhibitors.

Both above-mentioned WO publications disclose also the use of specific pteridine derivates in the treatment of patholigically low blood pressure, in particular septic shock and combined with cytokines in tumor therapy and in transplant-rejection diseases.

Although some of these pteridine derivates are claimed as potentially active for the treatment of transplant-rejection diseases, direct evidence for their effectiveness is lacking. Overall there still is a need for specific and highly active immunosuppressive compounds, in particular immunosupressive compounds active in the cosignal pathway.

A first object of the invention is to provide a pharmaceutical composition having high immunosuppressive activity. Another object of the invention is to provide a combined immunosuppressive preparation which causes a superadditive effect, comprising a pteridine derivate of the invention and other known immunosuppressants.

Another further object of the invention is to provide immunosuppressive compounds, which are active in a minor dose, in order to decrease the considerable treatment costs.

Known immunosuppressive compounds are for example cyclosporine A, subsituted xanthines, tacrolimus (FK 506), rapamycine (RPM), leflunomide, mofetil, adrenocortical steroids, cytotoxic drugs and antibody preparations.

The immunosuppressive effect of cyclosporine A (CyA) is already known since 1972. However, due to its nephrotoxicity and several other side effects CyA has not been able to establish itself as the optimal and final drug of choice.

Methylxanthines, for example pentoxifylline (PTX), are known having immunosuppressive effects in vitro.

Recently (Lin Y. et al, Transplantation 63 (1997) it has been found that the co-medication of an immunosuppressive compound such as cyclosporine A (CyA) or FK506 or RPM (rapamycine) with a methylxanthine derivative, in particular A802715 (7-propyl-1(5-hydroxy-5-methylhexyl)-3-methylxanthine) leads to a superadditive increase in the immunosuppressive action.

Likewise, other substituted, in particular substituted 8-phenylxanthines have been found to possess immunosuppressive effects in vitro (application EP 98.201323.7).

The present invention relates in particular to the application of a group pteridine derivates and their pharmaceutical salts, possessing unexpectedly desirable pharmaceutical properties, i.c. are higly active immunosuppressive agents, are usefull in the treatment in transplant rejection and/or in the treatment of inflammatory deseases.

The invention demonstrates the immunosuppressive effects of pharmaceutical compositions for the treatment of autoimmuno disorders or of transplant-rejections comprising one or more pteridine derivatives of the above formula (I) or salts thereof.

Experimental Part

2-amino-4-n-pentyloxy-6-styrylpteridine (1).

A mixture of 2-amino-6-chloro-4-n-pentyloxypteridine [1] (1.5 g, 5.6 mmoles) palladium acetate (63 mg, 0.28 mmoles), tri-o-tolylphosphane (682 mg, 2.24 mmoles), cuprous iodide (53 mg, 0.28 mmoles), styrene (1,3 ml., 11.3 mmoles) and triethylamine 3.1 ml, 22 mmoles) was stirred in dry acetonitrile (50 ml.) under reflux for 90 hours. It was evaporated and the residue purified by silica gel column chromatography with $CHCl_3$. The product fraction was evaporated to give 1.37 g (72%) of an orange powder. Recrystallization from EtOAc/hexane. M.p. 127-128°C.

2-Amino-6-(1,2-dibromophenethyl)-4-n-pentyloxypteridine (2).

To a solution of 1 (1.0 g, 2.94 mmoles) in chloroform (50 ml.) was added a 2 M bromine solution in chloroform (2.2 ml., 4.4 mmoles) and then the mixture stirred at room temperature for 7 hours. It was diluted with chloroform (50 ml.), washed with a saturated aqueous Na_2SO_3 solution (100 ml.) and dried over Na_2SO_4. It was evaporated, the residue treated with little toluene, filtered, washed with ether and dried in a vacuum desiccator to give 0.84 g (57%) yellow powder.

2-Amino-4,7-dimethoxy-6-styrylpteridine (3).

A suspension of 2 (0.3 g, 0.6 mmoles) is abs. Methanol (10 ml.) was treated with 1 M methanolic sodium methoxide (3 ml., 3 mmoles) and then refluxed for 4 hours. It was diluted with chloroform (100 ml.), washed with saturated aqueous NH_4Cl solution and water and then the solution dried over Na_2SO_4. The filtrate was evaporated and the residue purified by silica gel column chromatography in chloroform. The product fraction was evaporated to give 50 mg. (26%) of a yellow powder, M.p. 197-198°C.

O^4-Methyl-biopterin (4).

To a solution of N^2, 1',2'-O-triacetyl-biopterin (1,0 g; 2.75 mmoles), triphenylphosphane (12,08 g, 4.13 mmoles) and methanol (0.15 ml., 3.7 mmoles) in dry dioxane (30 ml.) was added diisopropyl azodicarboxylate (0.81 g, 4.11 mmoles) and after stirring for 1.5 hours at room temperature evaporated to dryness. The residue was purified by silica gel column chromatography eluting with EtOAc/$CHCl_3$ (1:4). The product fraction was evaporated and dried in vacuum to give 0.4 g (38%) of N^2, 1',2'-O-triacetyl-O^4-methylbiopterin.

Deacetylation of the reaction product (0.28 g, 0.74 mmoles) was done by stirring in abs. Methanol (20 ml.) and triethylamine (4 ml.) for 24 hours. Evaporation to dryness, treatment of the residue with ether, filtration and drying gave 0.172 g (83%) of 4. M.p. 160-161°C (Decomp.).

<u>General procedure for the synthesis of 2,4-diamino-6-arylpteridines (5, 7, 8, 9)</u>

A suspension of 2,4,5,6-tetraaminopyrimidine dihydrochloride (2.13 g, 0.01 moles) in methanol (100 ml.) was heated to boiling and then a solution of the arylglyoxalmonoxime (phenylglyoxalmonoxime [2], p-methylphenylglyoxalmonoxime [3], p-methoxyphenylglyoxalmonoxime [4], p-chlorophenylglyoxalmonoxime [5] (0.015 moles) in methanol (20 ml.) added dropwise within 30 min. It was heated under reflux for 2 hours forming a precipitate. After cooling was neutralized by conc. ammonia to pH 8 with stirring. The precipitate was collected, washed with methanol and ether and dried in the oven at 100°C. Yield: 85-95%. The reaction product is usually chromatographically pure. Recrystallization can be achieved from DMF.

<u>2,4-Diamino-7-methyl-6-phenylpteridine (6).</u>

Analogous to the preceding procedure using α-hydroximinopropiophenon. Yield: 70%.

General procedures for the synthesis of 4-amino-6-aryl-2-ß-hydroxyethylaminopteridines (10, 11,12)

A suspension of 4,5,6-triamino-2-ß-hydroxyethylaminopyrimidine trihydrochloride (2.93 g, 0.01 moles) in methanol (60 ml.) was heated under reflux and then a solution of the aryl-glyoxalmonoxime (0.015 moles) in methanol (15 ml.) added dropwise. After reflux for 2 hours and cooling was neutralized to pH 9 with conc. Ammonia to give a yellow precipitate. Yield: 90%.

2-amino-4-hydroxylamino-6-p-methoxyphenylpteridines (13).

A suspension of 2,5,6-triamino-4-methoxypyrimidine dihydrochloride (1 g, 4 mmoles) in methanol (40 ml.) was heated to boiling and then a solution of phenylglyoxalmonoxime (1 g, 6.6 mmoles) in methanol (10 ml.) added dropwise. A clear solution is obtained from which on reflux for 2 hours a precipitate separated out. The solid was filtered off (hydrochloride salt), suspended in water (30 ml) and then neutralized to pH 8 by conc. ammonia. The precipitate was collected, washed with water and ethanol and dried at 100°C to give a yellow powder. Yield: 0.84 g (82%).

2,4-diamino-6-bromomethylpteridine (6).

A suspension of 2,4,5,6-tetraaminopyrimidine trihydrobromide (3.0 g, 0.01 moles) in methanol (60 ml) was heated to reflux and then a solution of ß-bromopyruivaldoxime (0.015 moles) in methanol (30 ml) added dropwise within 10 min. The resulting yellow solution was refluxed for 30 min., then cooled to room temperature and neutralized by conc. ammonia to pH 8. The yellow precipitate was collected, washed with little methanol and ether and dried in a vacuum desiccator. Yield: 88%.

General procedure for 2,4-diamino-6-alkoxymethyl-(17,18) and -6-aminomethylpteridines (19,20).

To a mixture of dimethylacetamide (DMA) (30 ml) and the appropriate alcohol (ß-methoxyethanol, n-decanol) (5 ml) was added sodium hydride (1 g, 80%) and after stirring for 1 hour 2,4-diamino-6-bromomethylpteridine (1 g) added. Stirring was continued at room temperature for 6 hours, then diluted with H_2O (100 ml) and kept in the icebox for 2 days. The precipitate was collected and recrystallized from EtOH/ conc. NH_3 (16:1). Yield: 50%.

An analogous reaction takes place with amines (dimethylamine in ethanol, benzylamine) (0.04 mmoles) in DMA (20 ml) and 2,4-diamino-6-bromomethylpteridine (2.55 g, 0.01 moles). Yield: 50-60%.

General procedures for the synthesis of 2,6-diamino-4-dialkylamino-5-p-chlorophenylazopyrimidines.

A solution of 2,6-diamino-4-dialkylamino-5-p-chlorophenylazopyrimidine [7] (5.0 g, 16.6 mmoles) in DMF (50ml) and the appropriate amine (dimethylamine in ethanol (50%), diethylamine, di-n-propylamine, dibenzylamine, morpholine, piperidine, pyrrolidine, piperazine, N-methylpiperazine) (10.0 g) was heated in an oilbath to 70°C for 5 hours. Then water (50 ml) was added, cooled and the yellow precipitate collected, washed with water and dried. Recrystallization from EtOH or DMF/water. Yield: 55-90%.

General procedure for the synthesis of 2,5,6-triamino-4-dialkylaminopyrimidines.

A suspension of 2,6-diamino-4-dialkylamino-5-p-chlorophenylazopyrimidine (3.28 g, 10 mmoles) in methanol (70 ml) and conc. ammonia (10 ml) was reduced in a shaking apparatus under H_2 atmosphere in presence of Raney nickel catalyst (3.5 g) for 2 days. The catalyst was filtered off under argon atmosphere and then the filtrate evaporated in vacuo to dryness.

The residue was treated with ether to remove the p-chloroaniline, filtered and then the solid stirred in methanolic HCl (10%, 50 ml) overnight. The dihydrochloride salt was collected and dried in a vacuum desiccator over KOH. Yield: 85-90%.

General procedure for the synthesis of 2-amino-4-dialkylamino-6-arylpteridines (14-16, 21-49)

To a boiling solution of the 2,5,6-triamino-4-dialkylaminopyrimidine dihydrochloride salt (5 mmoles) in MeOH (20 ml) was added a solution of the arylglyoxalmonoxime (7.5 mmoles) in MeOH (10 ml) dropwise and then the mixture heated under reflux for 3 hours. After cooling the suspension or solution was made alkaline by conc. ammonia to pH 9 and the resulting precipitate filtered off, washed with water and dried. Recrystallization was done from EtOH and DMF/H_2O, respectively, to give a yellow solid. Yield: 50-85%.

2-amino-4-benzylamino-6-p-methoxyphenylpteridine (50).

Analogous to the preceding procedure from 2,5,6-triamino-4-dialkylaminopyrimidine dihydrochloride and p-methoxyphenylglyoxalmonoxime. Yield: 68%.

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Materials and methods

Various models may be used for testing an immunosuppressive effect. In vivo, for example, different transplantation models are available. They are strongly influenced by different immunogenicities, depending on the donor and recipient species used and depending on the nature of the transplanted organ. The survival time of transplanted organs can thus be used to measure the suppression of the immune response. In vitro, there exist also various models. The most used are lymphocyte activation tests. Usually activation is measured via lymphocyte proliferation. Inhibition of proliferation thus always means immunosuppression under the experimental conditions applied. There exist different stimuli for lymphocyte activation:

- coculture of lymphocytes of different species (MLR = mixed lymphocyte reaction): lymphocytes expressing different minor and major antigens of the HLA-DR type (= alloantigens) activate each other non-specifically.
- CD3 assay: here there is an activation of the T-lymphocytes via an exogenously added antibody (OKT3). This antibody reacts against the CD3 molecule located on the lymphocyte membrane. This molecule has a costimulatory function. The interaction anti-CD3 (= OKT3)-CD3 results in T-cell activation which proceeds via the Ca^{2}+/calmodulin/calcineurin system and can be inhibited by CyA.
- CD28 assay: here specific activation of the T-lymphocyte goes also via an exogenously added antibody against the CD28 molecule. This molecule is also located on the lymphocyte membrane, and delivers strong costimulatory signals. This activation is Ca^{2}-independent and thus cannot be inhibited by CyA.

Reagents

All derivatives were dissolved in 0.5 ml DMSO and further diluted in culture medium before use in in vitro experiments. The culture medium consisted of RPMI-1640 + 10% FCS.

Mixed Lymphocyte Reaction

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized peripheral blood by density gradient centrifugation over Lymphoprep (Nycomed, Maorstua, Norway). Allogeneic PBMC or EBV-transformed human B cells [RPMI1788 (ATCC name CCL156)] which strongly express B7-1 and B7-2 were used as stimulator cells after irradiation with 30 Gy. MLR was performed in triplicate wells. After 5 days incubation at 37°C, 1 μCi [^{3}H]-thymidine was added to each cup. After a further 16 hours incubation, cells were harvested and counted in a ß-counter.

The percent suppression of proliferation by drugs was counted using the formula

$$\text{Percent inhibition} = \frac{(\text{cpm}+\text{drugs})-\text{cpm Cult. Med}}{(\text{cpm}-\text{drugs})-\text{cpm Cult. Med.}} \times 100$$

T cell purification

T cells were purified by removing non-T cells. Briefly, monocytes were removed by cold agglutination. The resulting lymphoid cells were further purified by a cell enrichment immunocolumn [Cellect Human T (Biotex, Edmonton, Alberta, Canada)] by a process of negative selection. More than 95% of the B cells were removed with this procedure. After depletion, the resulting T cell preparation was highly purified explaining these cells could not be activated by PHA or rIL-2 alone at concentrations capable of stimulating RBMC prior to deletion.

Measurements of T cell proliferations induced by anti-CD3 mAb + PMA or anti-CD28 mAb + PMA

Highly purified T cells (10^6/ml) were stimulated by immobilized anti-CD3 ár anti-CD28 mAb in the presence of PMA. Anti-CD3 mAb (CLB-CD3; CLB, Amsterdam, The Netherlands) were fixed on the 96-microwell plates by incubating the wells with 50 µl of mAb solution (1/800 dilution in culture medium). Anti-CD28 mAb (CLB-CD28; CLB, Amsterdam, The Netherlands) 50 µl (1/650 dilution in culture medium) was added directly to the wells. Further, 20 µl PMA (Sigma, St. Louis, MO, USA) solution (final concentration: 0.5 ng/ml) was added. Subsequently, 20 µl of immunosuppressants were added by serial dilution in triplicate wells. Finally 100 µl of the T cell suspension (10^6/ml) was added. After 48-hour incubation at 37°C in 5% CO_2 20 µl BrdU (100 µM solution) (Cell Proliferation Elisa, Boehringer-Mannheim Belgium) was added to each well. After a further overnight incubation the T cell proliferation was measured using a colorimetric immunoassay for qualification of cell proliferation based on measurements of the incorporation of BrdU during DNAsynthesis. The optical density (OD) was measured by a Behring EL311 plate reader at 450 nm (reference wavelength: 690 nm). The percent suppression of proliferation by drugs was counted using the formula:

$$\text{Per cent inhibition} = \frac{(\text{OD}+\text{drugs})-(\text{OD Cult. Med.})}{(\text{OD}-\text{drugs})-(\text{OD Cult. Med.})} \times 100$$

<u>In vitro immunosuppressive effect of Pteridine Derivatives as measured with the MLR and with tests involving polyclonal T cell proliferation induced by anti-CD3 mAb + PMA or anti-CD28 mAb + PMA</u> (table II)

- Table II shows the IC50 values of the various substances in the MLR. The IC50 value represents the lowest concentration of the substances that resulted in a 50% suppression of the MLR.

- These concentrations are divided into for subranges i.e.
  - 0 stands for concentrations of at least 151μM,
  - \+ stands for concentrations 16-150 μM,
  - ++ stands for concentrations 1-15 μM,
  - +++ stands for concentrations lower than 1 μM,

- Column III shows the IC50 value of the various substances for the anti-CD3 mAb + PMA pathway and row IV the IC50 values of the various substances for the anti-CD28 mAb + PMA pathway.
- As a comparison the values of other immunosuppressants: CsA, FK506, Rapamycin, Leflunomide and Mycophenolic acid methatroxate (MTX) and 5-Fluoro-uracil (5-FU) in table III are given as well.

First, most of the pteridine classes (I) according to the invention contain compounds with a clear suppressive effect in the MLR (mixed lymphocyte reaction). The MLR is considered as an in vitro analogue of the transplant rejection as it is based on the recognition of allogeneic MHC (major histocompatibility antigens) on the stimulator leucotyes, by responding lymphocytes. Various established immunosuppressive drugs are known to suppress the MLR, and were also shown in this description.

From these data it can be deduced that the pteridine derivatives are effective in clinical situations where other immunosuppressants are active as well.

These include the prevention and/or treatment of organ transplant rejection, the prevention and/or treatment of both rejection and the occurrence of graft-versus-host-disease after BM transplantation; the prevention and/or treatment of autoimmune diseases including diabetes mellitus, multiple sclerosis, glomerulonephritis, rheumatoid arthritis, psoriasis systemic diseases such as vasculitis; scleroderma, polymyositis, autoimmune endocrine disorders (thyroiditis), ocular diseases

(uveitis), inflammatory bowel diseases (Crohn's disease, colitis ulcerosa), autoimmune liver diseases (autoimmune hepatitis, primary biliary cirrhosis) autoimmune pneumonitis and auto-immune carditis.

Whereas cyclosporine A and FK506 are only active in the anti-CD3 + PMA test, the pteridine derivatives according to the invention were active, not only in the anti-CD3 + PMA but also in the anti-CD28 + PMA test. It has been shown that the latter is Ca-calmodulin resistant, and resistant to CsA and FK506. The anti-CD28 + PMA pathway has also been called the cosignal pathway and is important to induce energy and even tolerance in T cells. Moreover, representative compounds have been found to be active in an whole blood assay.

Under the term "organ" in the description is understood all organs or parts of organs (even several) in mammals, in particular humans, for example kidney, heart, skin, liver, muscle, cornea, bone, bone marrow, lung, pancreas, intestine or stomach.

After organ transplantation, rejection of the transplanted organ by the recipient occurs (host-versus-graft reaction). After bone marrow transplantation, also rejection of the host by the grafted cell may occur (graft-versus-host reaction). Rejection reactions mean all reactions of the recipient body or of the transplanted organ which in the end lead to cell or tissue death in the transplanted organ or adversely affect the functional ability and viability of the transplanted organ or adversely affect the functional ability and viability of the transplanted organ or the recipient. In particular, this means acute and chronic rejection reactions.

Auto-immune disorders include, inter alia, systemic lupus erythematosus, rheumatoid arthritis, psoriasis, pemphigus, atopic dermatitis, myositis, multiple sclerosis, nephrotic syndrome (in particular glomerulonephritis), ulcerative colitis or juvenile diabetes.

An additive or synergetic effect of pteridine derivatives and other immunosuppressants may be anticipated. This may be especially, although not exclusively the case for combinations with CyA or FK 506 as the latter are not suppressive in the aCD28 pathway of T cell activation (table III) whereas most Ptedridine derivatives are.

The invention further relates to the use of cyclosporin A or FK506 or Rapamycine and at least one pteridine derivative according to the invention for the production of a pharmaceutical for inhibiting the replication of viruses such as picorna-, toga-, bunya-, orthomyxo-, paramyxo-, rhabdo-, retro-, arena-, hepatitis B-, hepatitis C-, hepatitis D-, adeno-, vaccinia-, papilloma-, herpes-, varicella-zoster-virus or human immunodeficiency virus (HIV); or for treating of cancer such as lung cancers, leukaemia, ovarian cancers, sarcoma, Kaposi's sarcoma, meningioma, colon cancers, lymp node tumors, glioblastoma multiforme, prostate cancers or skin carcinoses.

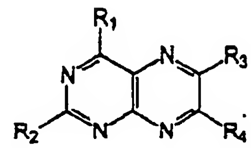
The invention further relates to the use of cyclosporin A or FK506 or rapamycin and at least one pteridine derivative of the general formula (I) for the production of a pharmaceutical for the treatment of human after organ transplantation or of (auto)immune disorders.

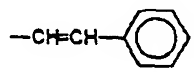
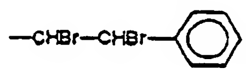
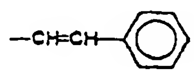
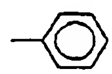
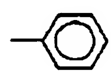
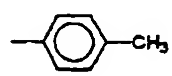
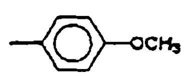
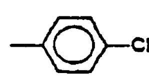
Hence, the advantage to associate pteridine with other immunosuppressants may be that, first, the therapeutic spectrum of action of the individual components is quantitatively and qualitatively broadened. Secondly that it allows, by means of a dose reduction without reduced efficacy but with increased safety, that the treatment of immune disorders which were hitherto no indication for immunosuppressive therapy as a result of side effects may be considered. At the same time, the therapy costs can be decreased to an appreciable extent.

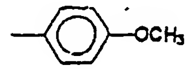
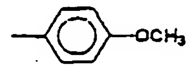
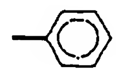
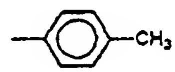
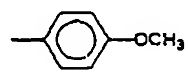
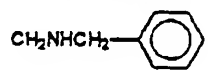
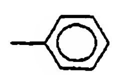
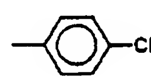
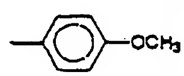
As a comparison, known pteridine derivatives are submitted to the same test conditions as the pteridine derivatives of the invention. These compounds and the results thereof are given in table IV and show no particular immunosuppressive activity.

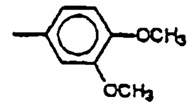
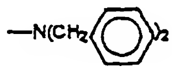
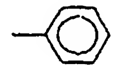
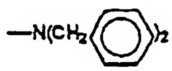
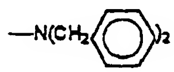
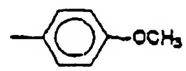
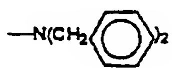
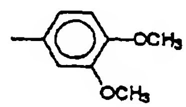
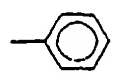
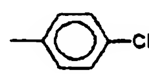
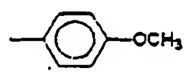
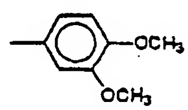
As been stated above the invention also relates to new pteridine derivatives as such, in particular the compounds 1,2,3,6, 14-16 and 21-50.

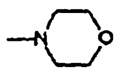
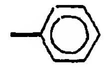
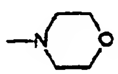
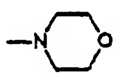
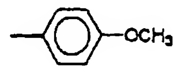
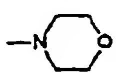
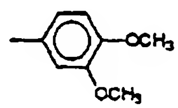
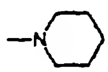
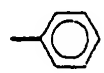
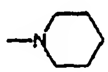
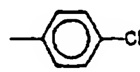
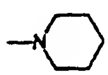
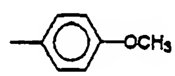
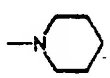
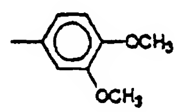
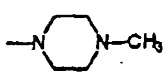
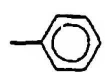
Table I

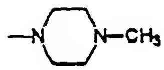
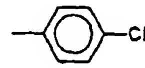


n°	R_1	R_2	R_3	R_4
1	OC_5H_{11}	NH_2		H
2	OC_5H_{11}	NH_2		H
3	OC_5H_{11}	NH_2		OCH_3
4	OCH_3	NH_2	—CHOH—CHOH—CH_3	H
5	NH_2	NH_2		H
6	NMe_2	NH_2		CH_3
7	NH_2	NH_2		H
8	NH_2	NH_2		H
9	NH_2	NH_2		H

n°	R_1	R_2	R_3	R_4
12	NH_2	NH-CH_2CH_2OH		H
13	NHOH	NH_2		H
14	NMe_2	NH_2		H
15	NMe_2	NH_2		H
16	NMe_2	NH_2		H
17	NH_2	NH_2	$CH_2OCH_2CH_2OCH_3$	H
18	NH_2	NH_2	$CH_2O(CH_2)_6CH_3$	H
19	NH_2	NH_2		H
20	NH_3	NH_2	$CH_2N(CH_3)_2$	H
21	NEt_2	NH_2		H
22	NEt_2	NH_2		H
23	NEt_2	NH_2		H

n°	R_1	R_2	R_3	R_4
24	NEt_2	NH_2		H
25		NH_2		H
26		NH_2		H
27		NH_2		H
28		NH_2		H
29	$—N(CH_2CH_2CH_3)_2$	NH_2		H
30	$—N(CH_2CH_2CH_3)_2$	NH_2		H
31	$—N(CH_2CH_2CH_3)_2$	NH_2		H
32	$—N(CH_2CH_2CH_3)_2$	NH_2		H

n°	R_1	R_2	R_3	R_4
33		NH_2		H
34		NH_2		H
35		NH_2		H
36		NH_2		H
37		NH_2		H
38		NH_2		H
39		NH_2		H
40		NH_2		H
41		NH_2		H

42		NH_2		H

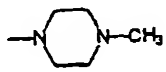
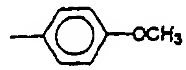
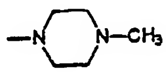
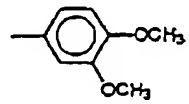
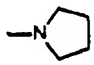
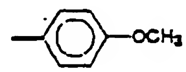
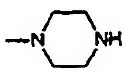
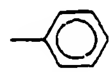
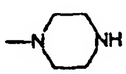
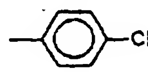
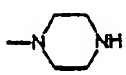
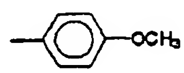
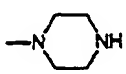
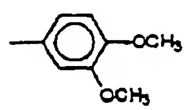
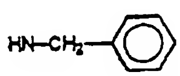
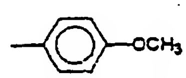
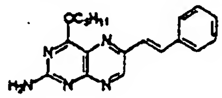
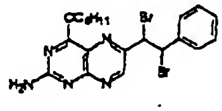
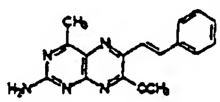
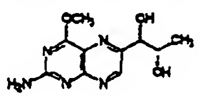
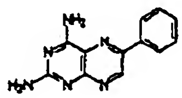
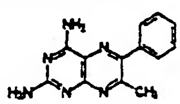
n°	R_1	R_2	R_3	R_4
43		NH_2		H
44		NH_2		H
45		NH_2		H
46		NH_2		H
47		NH_2		H
48		NH_2		H
49		NH_2		H
50		NH_2		H

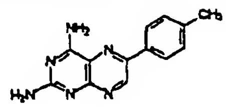
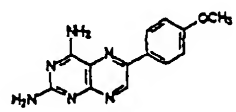
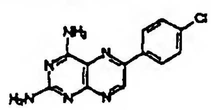
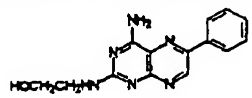
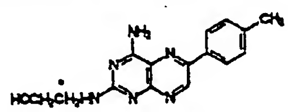
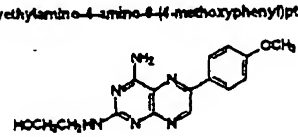
Table II

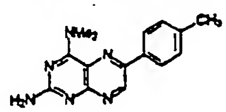
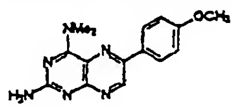
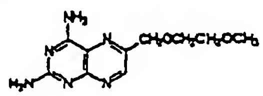
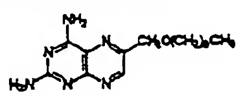
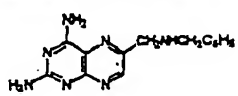
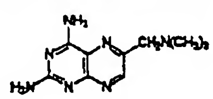
IC$_{50}$ in µM of pteridine derivative			
Compound n°	MLR	ACD3	aCD28
1	++	+	+
2	++	++	++
3	+	0	+
4	0	+	+
5	0	0	0
6	0	0	0
7	0	+	+
8	+	+	+
9	+	+	+
10	+	+	+
11	0	0	0
12	0	0	0
13	0	0	0
14	++	++	++
15	++	++	++
16	+	+	+
17	0	0	+
18	0	0	+
19	+	+	+

IC_{50} in µM of pteridine derivative			
Compound n°	MLR	ACD3	aCD28
20	0	0	+
21	++	+	+
22	+	+	+
23	++	+	+
24	+++	+++	+++
25	+	+	+
26	+	+	+
27	+	+	++
28	++	++	++
29	++	+	+
30	++	++	++
31	++	++	++

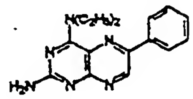
Compound n°	MLR	aCD3	ACD28
32	++	+++	+++
33	++	+	+
34	++	+	+
35	++	+	+
36	+++	+++	+++
37	++	+	+
38	++	+	+
39	++	++	++
40	+++	+++	+++
41	++	+	+
42	++	++	++
43	++	++	++
44	++	++	++
45	+	+	++
46	++	++	++
47	++	+	++
48	++	++	++
49	++	++	++
50	++	++	++

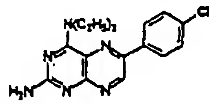
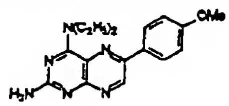
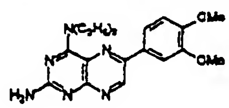
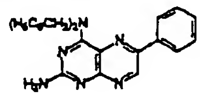
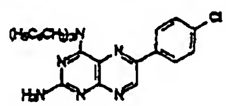
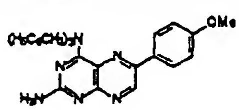
Compound n°		MLR	aCD3	ACD28
1	2-amino-4-pentoxy-6-styrylpteridine 	++	+	+
2	2-amino-4-n-pentoxy-6-(1,2-dibromo-2-phenylethyl)pteridine 	++	++	++
3	2-amino-4-methoxy-6-styryl-7-methoxypteridine 	+	0	+
4	2-amino-4-methoxy-6-(1,2-dihydroxypropyl)pteridine 	0	+	+
5	2,4-diamino-6-phenyl pteridine 	0	0	0
6	2,4-diamino-6-phenyl-7-methylpteridine 	0	0	0

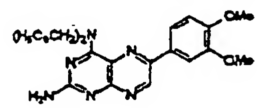
	Compound n°	MLR	AcD3	ACD28
7	2,4-diamino-6-(4-tolyl)pteridine 	0	+	+
8	2,4-diamino-6-(4-methoxyphenyl)pteridine 	+	+	+
9	2,4-diamino-6-(4-chlorophenyl)pteridine 	+	+	-
10	2-hydroxyethylamino-4-amino-6-phenylpteridine 	+	+	+
11	2-hydroxyethylamino-4-amino-6-(4-tolyl)pteridine 	0	0	0
12	2-hydroxyethylamino-4-amino-6-(4-methoxyphenyl)pteridine 	0	0	0

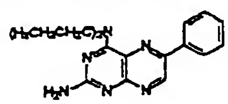
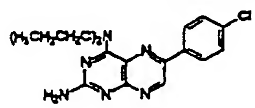
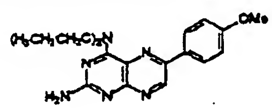
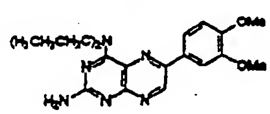
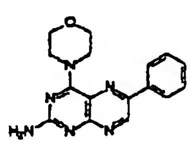
	Compound n°	MLR	ACD3	ACD28
15	2-amino-4-dimethylamino-6-(4-tolyl)pteridine 	++	++	++
16	2-amino-4-dimethylamino-6-(4-methoxyphenyl)pteridine 	++	++	++
17	2,4-diamino-6-methoxyethoxymethyl pteridine 	0	0	+
18	2,4-diamino-6-decyloxymethyl pteridine 	0	0	+
19	2,4-diamino-6-benzylaminomethyl pteridine 	+	+	+
20	2,4-diamino-6-dimethyl aminomethyl pteridine 	0	0	+

21 2-amino-4-diethylamino-6-phenylpteridine

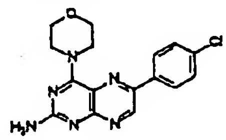


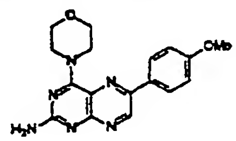
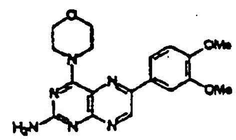
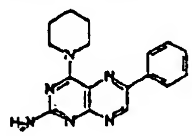
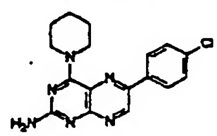
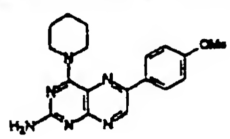
	Compound n°	MLR	ACD3	ACD28
22	2-amino-4-diethylamino-6-(4-chlorophenyl) pteridine 	+	+	+
23	2-amino-4-diethylamino-6-(4-methoxyphenyl) pteridine 	++	+	+
24	2-amino-4-diethylamino-6-(3,4-dimethoxyphenyl) pteridine 	+++	++	+++
25	2-amino-4-dibenzylamino-6-phenyl pteridine 	+	+	+
26	2-amino-dibenzylamino-6-(4-chlorophenyl) pteridine 	+	+	+
27	2-amino-4-dibenzylamino-6-(4-methoxyphenyl) pteridine 	+	+	++

28	2-amino-4-dibenzylamino-6-(3,4-dimethoxyphenyl) pteridine 	++	++	++

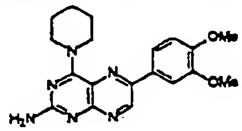
	Compound n°	MLR	ACD3	ACD28
29	2-amino-4-dipropylamino-6-phenylpteridine 	++	+	+
30	2-amino-4-dipropylamino-6-(4-chlorophenyl)pteridine 	++	++	++
31	2-amino-4-dipropylamino-6-(4-methoxyphenyl)pteridine 	++	++	++
32	2-amino-4-dipropylamino-6-(3,4-dimethoxyphenyl)pteridine 	++	+++	+++
33	2-amino-4-morpholino-6-phenylpteridine 	++	+	+

34 2-amino-4-morpholino-6-(4-chlorophenyl)pteridine

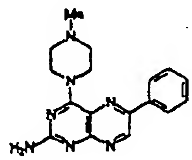
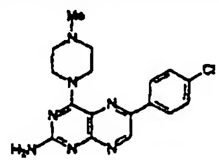
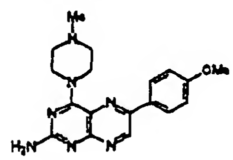
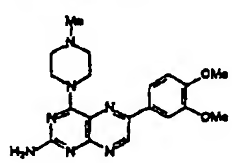
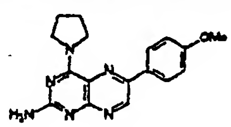


	Compound n°	MLR	ACD3	ACD28
35	2-amino-4-morpholino-6-(4-methoxyphenyl)pteridine 	++	+	+
36	2-amino-4-morpholino-6-(3,4-dimethoxyphenyl)pteridine 	+++	+++	+++
37	2-amino-4-piperidino-6-phenylpteridine 	++	+	+
38	2-amino-4-piperidino-6-(4-chlorophenyl)pteridine 	++	+	+
39	2-amino-4-piperidino-6-(4-methoxyphenyl)pteridine 	++	++	++

40 2-amino-4-piperidino-6-(3,4-dimethoxyphenyl)pteridine



+++ +++ +++

	Compound n°	MLR	ACD3	ACD28
41	2-amino-4-N-methylpiperazino-6-phenylpteridine 	++	+	+
42	2-amino-4-N-methylpiperazino-6-(4-chlorophenyl)pteridine 	++	++	++
43	2-amino-4-N-methylpiperazino-6-(4-methoxyphenyl)pteridine 	++	++	++
44	2-amino-4-methylpiperazino-6-(3,4-dimethoxyphenyl)pteridine 	++	++	++
45	2-amino-4-cyclopentylamino-6-(4-methoxyphenyl)pteridine 	+	+	++

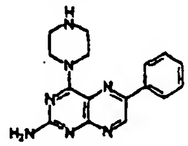
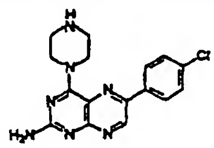
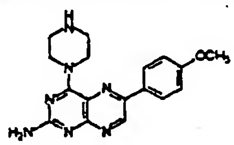
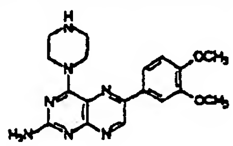
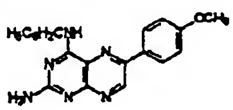
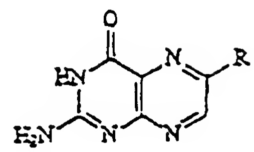
Compound n°	MLR	ACD3	AcD28
46 2-amino-4-piperazino-6-phenylpteridine 	++	++	++
47 2-amino-4-piperazino-6-(4-chlorophenyl)pteridine 	++	+	++
48 2-amino-4-piperazino-6-(4-methoxyphenyl)pteridine 	++	++	++
49 2-amino-4-piperazino-6-(3,4-dimethoxyphenyl)pteridine 	++	++	++
50 2-amino-4-benzylamino-6-(4-methoxyphenyl)pteridine 	++	++	++

Table III

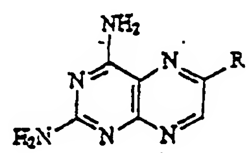
I.S.	IC50		
	Immunosuppressant		
	MLR	aCD3	aCD28
CyA	20 nM	50 nM	N.S.
FK506	1 nM	1 nM	N.S.
Rapamycin	1 nM	1 nM	1 nM
Leflunomide	25 μM	15 μM	20 μM
Mofetil	<0.5μM	50 nM	50 nM
MTX	10 μM	>200 μM	> 200 μM
5-FU		50μM	17 μM

N.S. = not suppressive even not in the highest
Concentration

Table IV



R	MLR	aCD3	aCD28
$CH_2OOCOCH_3$	0	0	0
$CH_2OC_6H_5$	0	0	0
CH_2NHCH_3	0	0	0
$CH_2N(CH_3)_2$	0	0	0
$CH_2S\ CH_3$	0	0	0



R	MLR	aCD3	aCD28
CH_2 S CH_3	0	0	0
CH_2 O $CH_6(CH_3)_2$	0	0	0
CH_2 O CH_3	0	0	0
CH_2 O $CH_2CH_2CH_3$	0	0	0
CH_2 NH CO CH $(CH_3)_2$	0	0	0